Microfabricated silicon biosensors for microphysiometry

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Abstract

Microphysiometers are biosensor devices that measure the metabolic rate of living cells by detecting the rate of extracellular acidification caused by a small number of cells. The cells are entrapped in a microvolume chamber, whose bottom surface is a silicon sensor chip. In a further miniaturization step, we have recently fabricated multichannel flow-through chips that will allow greater throughput and multiplicity. Microphysiometer technology can be applied to the detection of microorganisms. We describe the sensitive detection of bacteria and yeast. Further applications of microphysiometry to the characterization of microorganisms can be anticipated.

Introduction

The first silicon sensors were essentially extensions of purely electronic components to transducing components. A good example is the ion-sensitive field-effect transistor, first described in 1970 [1]. This transistor is an active electronic device coupled to an insulator/electrolyte interface which gives it a chemically dependent threshold voltage. This represents the electronic component point of view. Soon afterwards, however, a mechanical point of view became prominent as well, and was summarized in a well-known review paper by Peterson [2]. This development was made possible by the rapid progress in micromachining, and the realization that silicon as a material has favorable mechanical properties for many applications.

One can thus conceive of two aspects to silicon sensor technology: using the electronic aspect of silicon (e.g. all FET-based chemical sensors; various types of magnetotransistors), and using the mechanical properties (e.g. pressure sensors and accelerometers). Much of the recent most impressive progress in microsensor technology comes from combining these two aspects. A recent example of a commercial device that does this is a surface micromachined accelerometer with integrated circuitry [3].

Our work concerns the application of a silicon sensor chip for bioassays [4-7] and immunoassays [8-10]. In this case, the silicon chip also fulfills two functions: it is the chemical sensor, but it is also an important mechanical part of the fluid path needed to operate the system. We have described applications of silicon micromachining for our sensors [11]. It is the combination of these two properties of silicon which make it the material of choice for our application.

We have called the bioassay application of the silicon sensor a microphysiometer, which is a device which allows the measurement of the metabolic rate of cells [4]. Most of the applications we have described up to now are intended for mammalian cells [4–7]. In this paper, we will present an example of detection of microorganisms, namely bacteria and yeast, using the microphysiometer. Our preliminary work in that area concentrated on detecting *Escherichia Coli*, and measuring the effect of antibiotics on these bacteria [12]. The methods described in this paper may prove useful in a number of fields in which sensitive microbial detection and indentification are of significant importance. These include environmental, food, and industrial processes where small numbers of of contaminating microorganisms may be difficult to detect with existing methods but, after being allowed a period of growth, may have a significant impact on the ecosystem, patient, or product. In many cases the identity of these organisms must be determined in order to establish the source of the contamination and the likelihood of problems occurring (e.g. pathogenic capability or production of a toxin that causes food toxicity).

LAPS devices in microvolume chambers

The sensor device we use is called a Light-Addressable Potentiometric Sensor (LAPS) [13] (see Figure 1). It consists of an MIS or EIS capacitor which is illuminated by an AC-modulated light source, at a wavelength short enough to generate carriers in silicon, but also long enough to penetrate sufficiently. The typical wavelength we use is 940 nm. The illumination typically occurs at the backside of the device, so that the frontside is free to form one surface of the sensing chamber. When the capacitor is biased in inversion, the depletion region at the frontside surface collects light-generated carriers, generating an AC photocurrent. When the capacitor is biased in accumulation, there is no photocurrent. The resulting AC photocurrent/bias curves show a steep transition between these two states. We use the position of the inflection point in these curves to track changes in the potential at the insulator surface. Depending on the material used as the outer gate material of the LAPS, it can be used to detect pH, redox potential, or cations using an ion-selective membrane [13]. In all the applications described in this paper, we use a Si_3N_4 surface to detect pH changes. The measurement of the photocurrent/bias curves, and the calculation of the inflection point voltage is done with custom hardware and software.

In all our work to date, we have not focused on the LAPS as a stand-alone sensor, but rather on the combination of LAPS with another concept, namely microvolume reaction chambers. This consists of using the LAPS as part of a chamber with volume in the order of a microliter. Using such a small volume allows sensitive detection of any pH-changing reaction. According to the analysis of pH changes in a microvolume reaction chamber [14], the measured signal is a rate of pH change given by:

$$\frac{dpH}{dt} = \frac{R}{V \beta_V + S \beta_S}$$

(1)

where R is the rate at which a reaction produces H^+ or OH^- ions (in moles/second), β_V and β_S represent the volume and surface buffering capacity, respectively, V is the chamber volume, and S the surface area present.

This equation shows that the two important factors in the sensitivity of the system are the chamber volume, and the solution buffer capacity. In most cases, the surface buffer capacity is not dominant and can be ignored. Surface buffering, however, becomes the limiting factor when the volume is reduced below a certain point.

In the case of an immunoassay, an enzyme label is used to detect the analyte of interest, and R comes from an enzyme-catalyzed reaction [9,13]. In the case of a bioassay, the metabolism of living cells in the chamber causes the pH changes.

Measurement of cellular metabolism

Living cells constantly consume energy for a variety of purposes, such as maintaining chemical concentration gradients, mechanical motion or deformation, and the synthesis of compounds. This energy is stored in the form of adenosine triphosphate (ATP), that must therefore constantly be replenished. A variety of catabolic pathways exist in which nutrient compounds are broken down to provide energy in the form of ATP. We have analyzed these in detail in Ref. [15]. The two most important ones are glycolysis, which is the conversion of glucose to lactic acid without requiring the presence of oxygen, and aerobic respiration, which requires the presence of oxygen that enables a more complete metabolic breakdown of glucose, with CO_2 as the ultimate product. This carbon dioxide dissolves in water, and some of it is hydrated into carbonic acid. Thus, in both cases the end result of an energy-yielding metabolic pathway is an acidic substance. The metabolism of living cells therefore tends to acidify the extracellular environment. Table 1 gives a more complete list of energy producing pathways, and the amount of ATP and protons they generate. The method we use to detect cellular metabolism is the measurement of the rate of acidifaction it induces in the extracellular environment.

There are some important differences between microorganisms and mammalian cells when placed in a microphysiometer. Many more energy-producing pathways are available to most microorganisms. This means that the results will depend strongly on the carbon source. For instance, certain microorganisms have the ability to use pathways that alkalinize the environment rather than acidify it. Finally, microorganisms require less delicate treatment than mammalian cells, and can be entrapped with methods that would harm or kill mammalian cells.

In order to survive, cells must be bathed in a culture medium to supply them with nutrients, and keep the pH of the environment in a tolerable range. On average, a flow of this medium is needed to bring fresh nutrients and remove the acid byproducts of cellular metabolism. However, when there is a flow of medium in the microvolume chamber where the cells are, the chamber pH is determined by that of the medium. Thus, to measure the rate of extracellular acidification, the medium flow must be

periodically interrupted. In the absence of medium flow, the cells act as a source of protons, and Eq. (1) can be used to predict the resulting rate of pH change. It is seen that the measured signal is proportional to the acidification rate.

The measurement method consists of periodically turning the medium flow on and off, and measuring the acidification rate during the off periods. Figure 2 shows a diagram of the setup we use to carry out such a measurement, and Figure 3 shows an example of the result. The data are usually plotted as the acidification rate as a function of time. The main applications of this technique we have explored previously are the detection of receptor/ligand interactions [5], and in-vitro toxicology [16].

A multichannel, flow-through, microphysiometer chip

In all the embodiments of our technology which we have described previously, one silicon sensor chip is used in one flow chamber assembly, to constitute a single microphysiometer channel. Typically, eight channels are then combined in one table-top instrument.

Clearly, a silicon sensor chip with multichannel capability would be very desirable. However, as soon as several fluid streams are present in one chip, several new issues arise. There must be a separation between different flow channels, and a means to introduce cells into each chamber. This can be accomplished by etching channels into the surface of a silicon chip. Cells can then be introduced in all channels at once by coating a glass cover slip with a layer of adherent cells, and placing it over all the channels. This means, however, that entry and exit ports to these fluid channels cannot be at the top surface, since that is covered by the glass cover slip. The logical solution is to etch openings completely through the chip, with interfaces to the rest of the fluidics at the bottom of the chip.

Along these lines, we have designed and fabricated an eight-channel flow-through microphysiometer chip. Figure 4 shows the cross-section of one of the channels. The procedure to fabricate this chip starts with growing an oxide layer on a double-sided polished n-type silicon wafer, and patterning it to expose the frontside areas where the channels will be. The channel is then etched 100 μm deep in a wet anisotropic etchant solution. Next, openings are made on the backside for the flow-through holes, and anisotropically etched all the way through the wafer. The initial oxide layer is then stripped, leaving a bare patterned wafer. The process from then on is similar to the standard LAPS process described in [17]: essentially field oxidation, active area patterning, gate oxide, and LPCVD nitride deposition. One difference is that the backside now contains two gold electrodes: one makes contact to the silicon, and the other is on top of the field oxide, and is used as a controlling electrode.

Figure 5 shows the front and back sides of the completed 23 Y 23 mm chips. This chip combines in one unit all the chambers of a complete desktop microphysiometer. It could be used to make a much more compact eight-channel instrument, or several of these chips could be used to make an instrument with many more channels. This last option would be useful in applications such as drug discovery which require high throughput, and applications where redundancy is required to achieve very high reliability

performance. Another advantage of a multichannel micromachined chip is that many of the critical components of the system are combined in one chip, making the system easier and potentially cheaper to build.

Detection and characterization of microorganisms

Our initial experiments involving microbial cells used a chamber design which was different from that previously described [4,5] for eucaryotic cells. The metabolism of microbial cells was measured with a cell capsule which consisted of two fluid paths which passed through circular 0.45 µm pore polycarbonate membranes located above "reading areas" of a silicon chip (Fig. 6). The diameter of each of these membranes, and hence the boundaries of the cellular environment, was 700 µm. Before cells were introduced into one of these fluid paths (the other served as a negative control), a "background" acidification rate of 2.1 (SD of 2.8) µV/s was observed. The liquid passing through the chamber was OF medium and flow was on for 100 sec at a rate of 75 µl/min and off for 100 sec.

The bacterium *Escherichia coli* (ATCC 25922 cells) was grown at 37° C overnight in Trypticase soy broth, counted with a Petroff-Hauser direct cell counter, and then diluted into oxidation/fermentation (OF) medium (Difco Laboratories; Detroit, Michigan) at a concentration of 10^5 cells per ml. One tenth ml (10,000 cells) of this suspension was introduced into the cell chamber at 37° C. At this point the acidification rate was 3.1 (SD of 1.0) µV/s, which was not statistically different from the background acidification rate. However, when the fluid passing through the counting chamber was switched to a salts solution with very low buffering capacity, an acidification rate of 53.8 (S_D of 2.6) was observed (Fig 7). The time from introduction of cells until the beginning of these rate determinations was approximately 29 min.

Similarly, the yeast *Saccharomyces cerevisease* was grown overnight in Sabouraud dextrose medium (Difco) and diluted to 10^4 cells per ml in OF medium. One tenth ml of this suspension passing through the cell chamber at 25° C carried 1000 yeast cells into the chamber and yielded an acidification rate of 153 µV/s (S_D of 7.7), as shown in Fig. 8.

It should be noted that this particular cell chamber was designed to test the concept of microbial cell entrapment and was not specifically designed for optimum sensitivity. This design did demonstrate that a temporary substitution of one medium for another of lower buffering capacity (this switch was temporary because the latter was incapable of supporting growth) could greatly improve the ability to detect microbial metabolism. It is likely that a cell chamber constructed to take advantage of fluid switching capability but with a significantly lower cell chamber volume would represent a design with the capability for a sizable improvement in sensitivity.

In addition to detection, the microphysiometer has the possibility of characterizing the nature of the entrapped microorganisms. The response of different organisms to changes in the carbon source varies. The doubling time of an organism in a given growth medium can be measured in the microphysiometer, and is characteristic for that organism. Also, certain antibiotics are selective in inhibiting the growth of certain types of

microorganisms. It is possible to expose organisms to a sequence of different growth media, with and without antibiotics, to gain information about their nature, as we showed in [12].

Design of a micromachined, multichannel, microorganism-sensing chip

Given the flow-through chip for adherent eucaryotic cells we describe above, a design for a similar device for the entrapment and detection of microorganisms can be suggested easily. The key requirement is a flow channel that goes though a microorganism-entrapping membrane. In contrast to the chip of Fig. 4, this means a different chip must contain the inlet and outlet flow channel. Thus we suggest the concept shown in Fig. 9: two silicon chips, separated by a membrane. The lower chip contains the LAPS sensors, and has fluid inlets in the bottom. The function of the upper chip is to form the top of the microvolume chamber, collect the flow though the membrane, and guide it to an outlet. As before, a mechanical fixture applies enough pressure to hold these three components in place, and contains the fittings to interface the fluid connections with external pumps and valves.

One important issue in this design is to ensure that the flow in each channel goes as we expect, and does not leak underneath or through the membrane from one channel to the next. This potential problem can be handled in several ways. First, the membrane that we intend to use has directional openings which only go through, and not sideways (e.g., a track-etched 0.45 µm pore size polycarbonate membrane). Thus, the lateral flow resistance between neighboring channels is very high. To avoid lateral flow underneath the membrane, if pressure is not sufficient, a thin hydrophobic gasket material (silicone rubber, for instance) may be added.

Conclusions

We believe the microphysiometer is a powerful and general tool for detecting how living cells react to their environment, and for detecting components in the environment that affect living cells. We have only begun to explore these applications. In addition, there are many possibilities in applying this technology to the identification and characterization of microorganisms.

The advantages of integrating the silicon sensor with silicon micromachining technology are clear: integration allows multichannel sensors to be made, and miniaturization of the instrumentation. The direction of further developments will probably be the further integration of all critical components of the fluid path shown in Fig. 2 on the chip or close to it. The first candidate for further integration will be the valve which allows switching between two fluid streams, so that we can minimize the dead volume between the valve and the chamber.

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Carbon Source	Pathway	Reaction	ATP Yield	H^+ per ATP
Glucose	Glycolysis	glucose --> 2 lactate$^-$ + 2 H^+	2	1.000
Glucose	Respiration	glucose + 6 O_2 --> 6 HCO_3^- + 6 H^+	36	0.167
Glutamine	Respiration	glutamine + 9/2 O_2 + 3 H2O --> 5 HCO_3^- + 2 NH_4^+ + 3 H^+	27	0.111
Pyruvate	Respiration	pyruvate$^-$ + 5/2 O_2 + H_2O --> 3 HCO_3^- + 2 H^+	15	0.333

Table 1

Figure Captions

Figure 1. Diagram of basic LAPS setup. The electronics makes three analog connections to the sensor, beyond those to the LED: one to the bulk silicon, one to a controlling electrode, and one to the reference electrode.

Figure 2. Instrumentation schematic. Culture medium is pumped from a reservoir by a peristaltic pump, and passes through a debubbler/-degasser, a selection valve, the flow chamber, the reference electrode, and finally goes to a waste receptacle. The valve controls which of two pumped streams of medium enters the flow chamber (not shown). A personal computer controls the LAPS electronics, the pump, and the valve; it also manages data acquisition, analysis, and storage.

Figure 3. Example of a metabolic rate measurement. (A) pH vs. time. (B) The acidification rates resulting from the data in (A). Receptors on the cells were activated with an agonist midway through the period shown, causing a strong increase in acidification rate.

Figure 4. Cross-sectional diagram of one channel of a multichannel flow-through microphyiometer chip.

Figure 5. (A) Diagram of the frontside of the multichannel flow-through microphyiometer chip. (B) Photograph of the backside of the same chip, showing separate gold leads for backside contact and controlling electrode.

Figure 6. Diagram of cell chamber and flow path for bacterial detection

Figure 7. Acidification rates of E. coli in OF (low buffered) and salts solution (very low buffered) media

Figure 8. Acidification rates of yeast in OF medium (low buffered) and salts solution (very low buffered) media

Figure 9. Cross-sectional diagram of one channel of a conceptual flow-through microphyiometer chip for the entrapment and detection of bacteria.
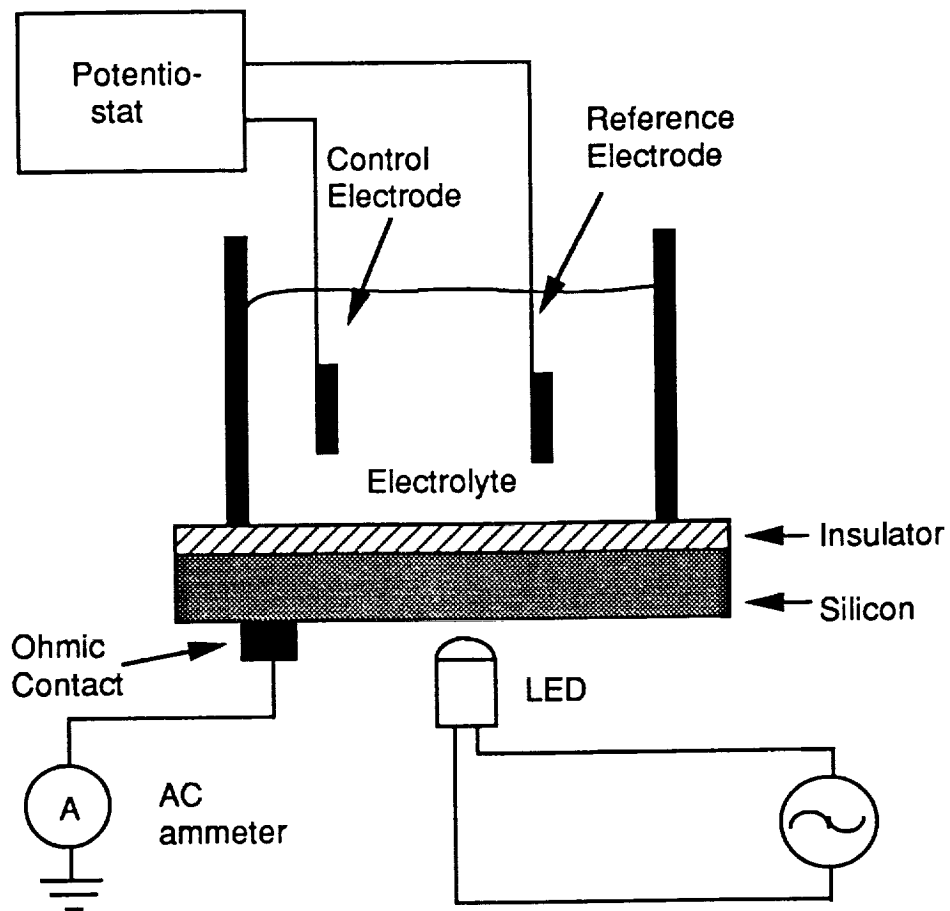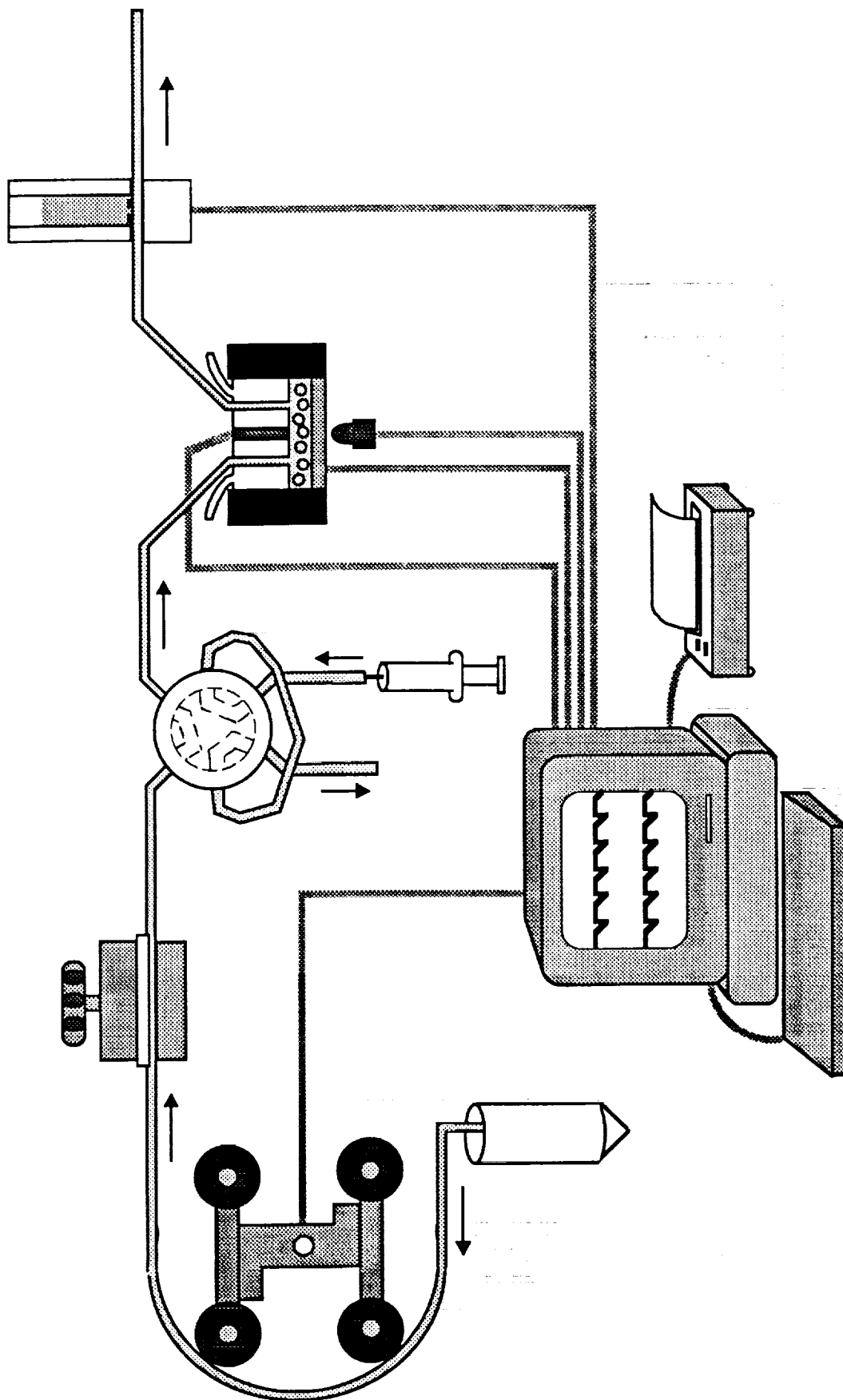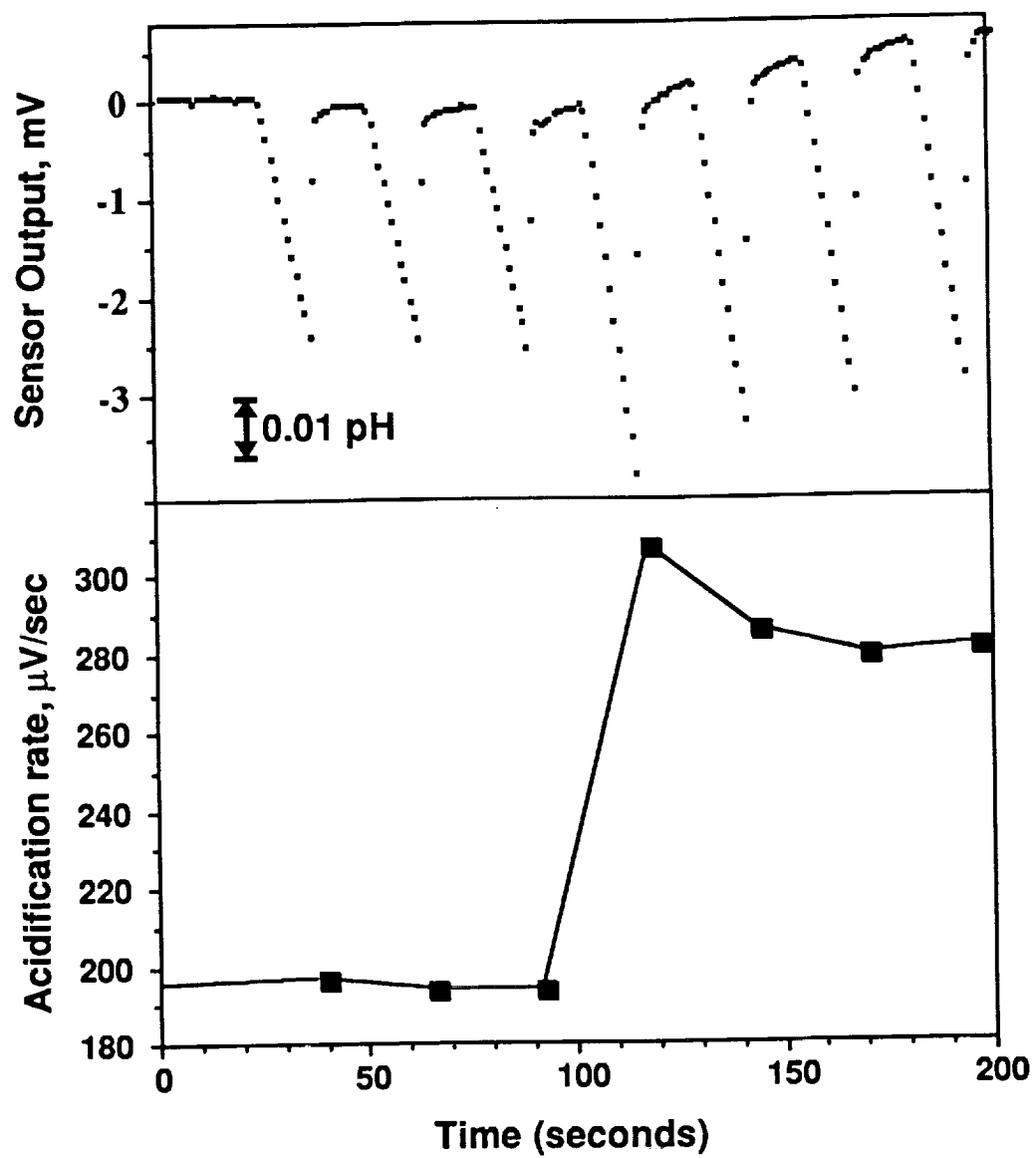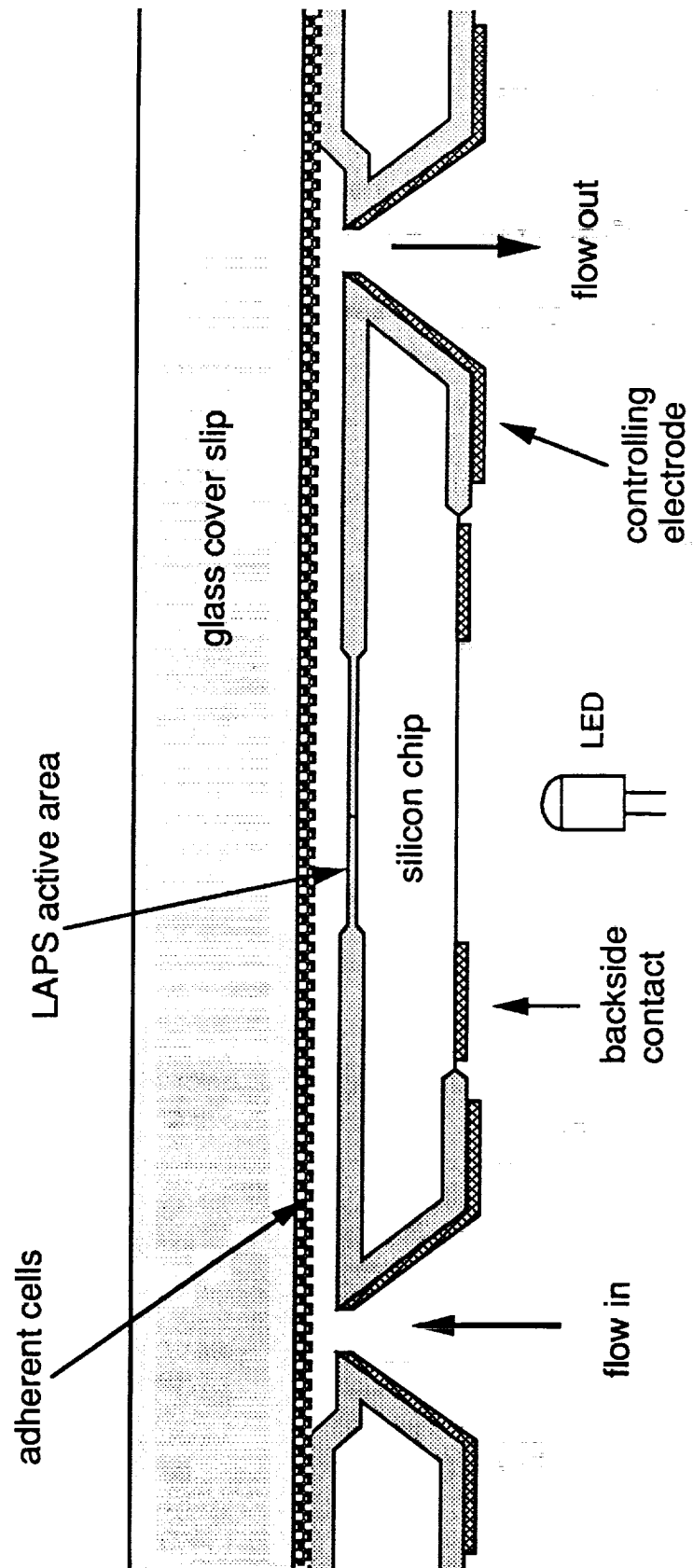
172

Figure 1.

Figure 2.

Figure 3.

adherent cells

LAPS active area

glass cover slip

silicon chip

backside
contact

controlling
electrode

flow in

flow out

LED

Figure 4.

176

Figure 5A.

Figure 5 B.

Fluid

Fluid

Controlling
electrode

Cells

o-ring

0.45 μm
membrane

Silicon

LED's

Fluid inlets

0.45 μm
Membrane

0.45 μm
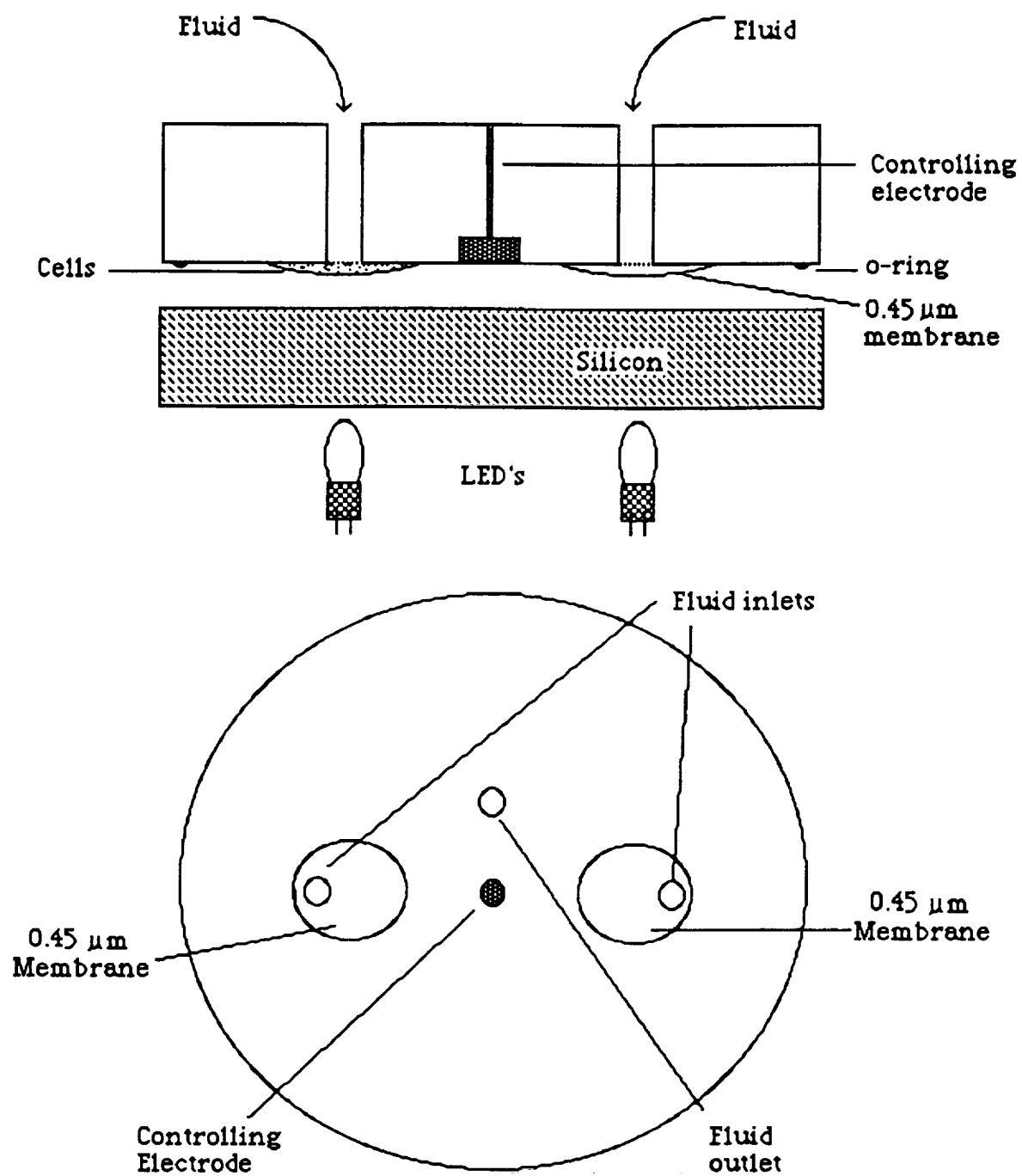Membrane

Controlling
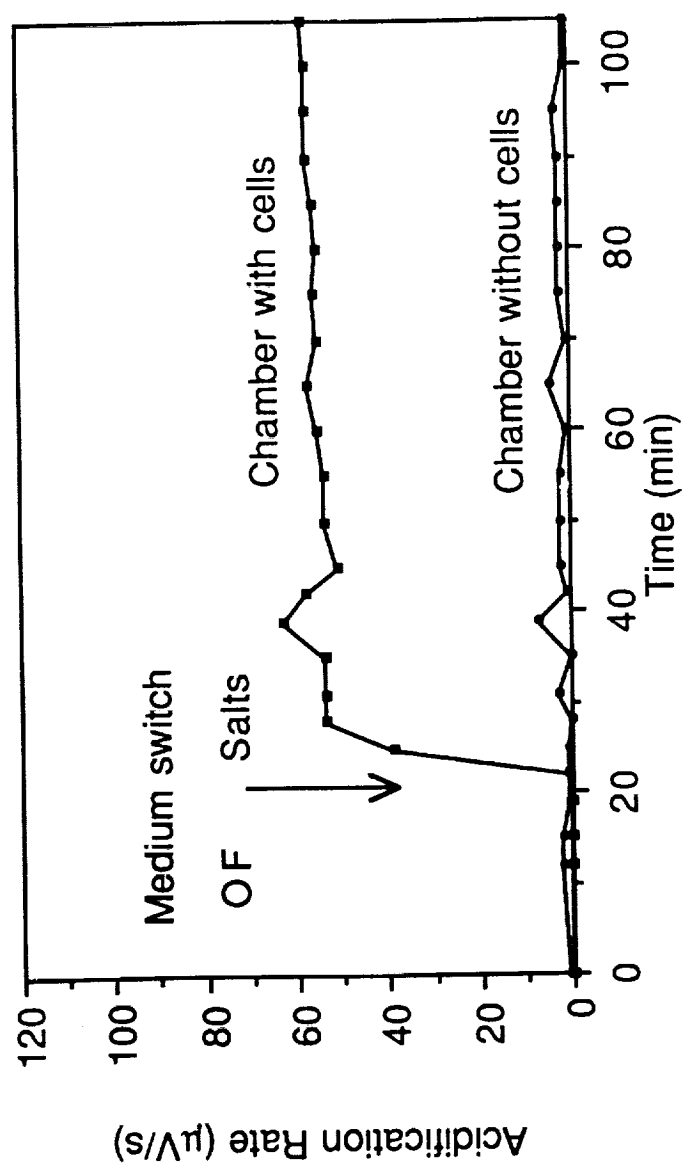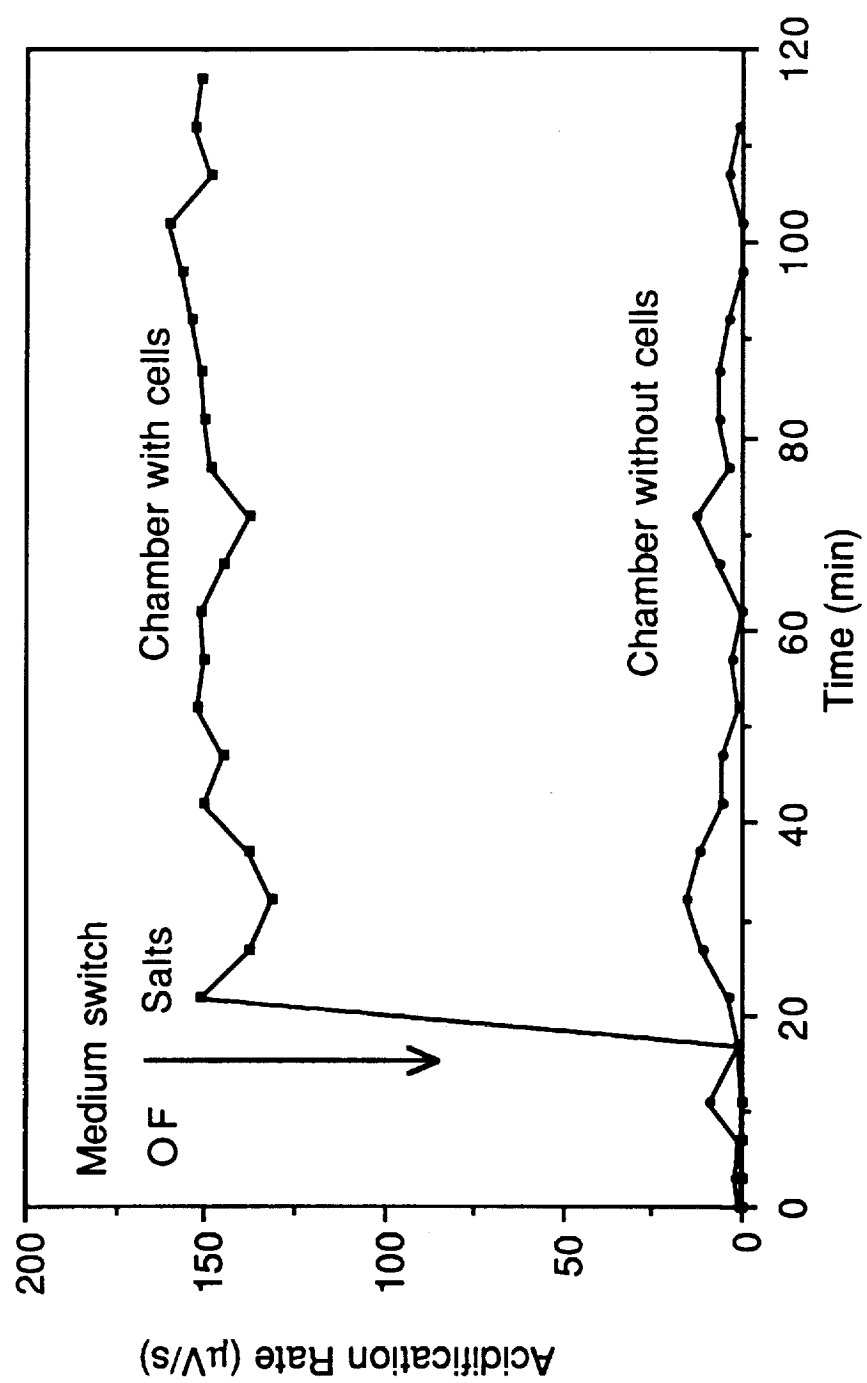Electrode

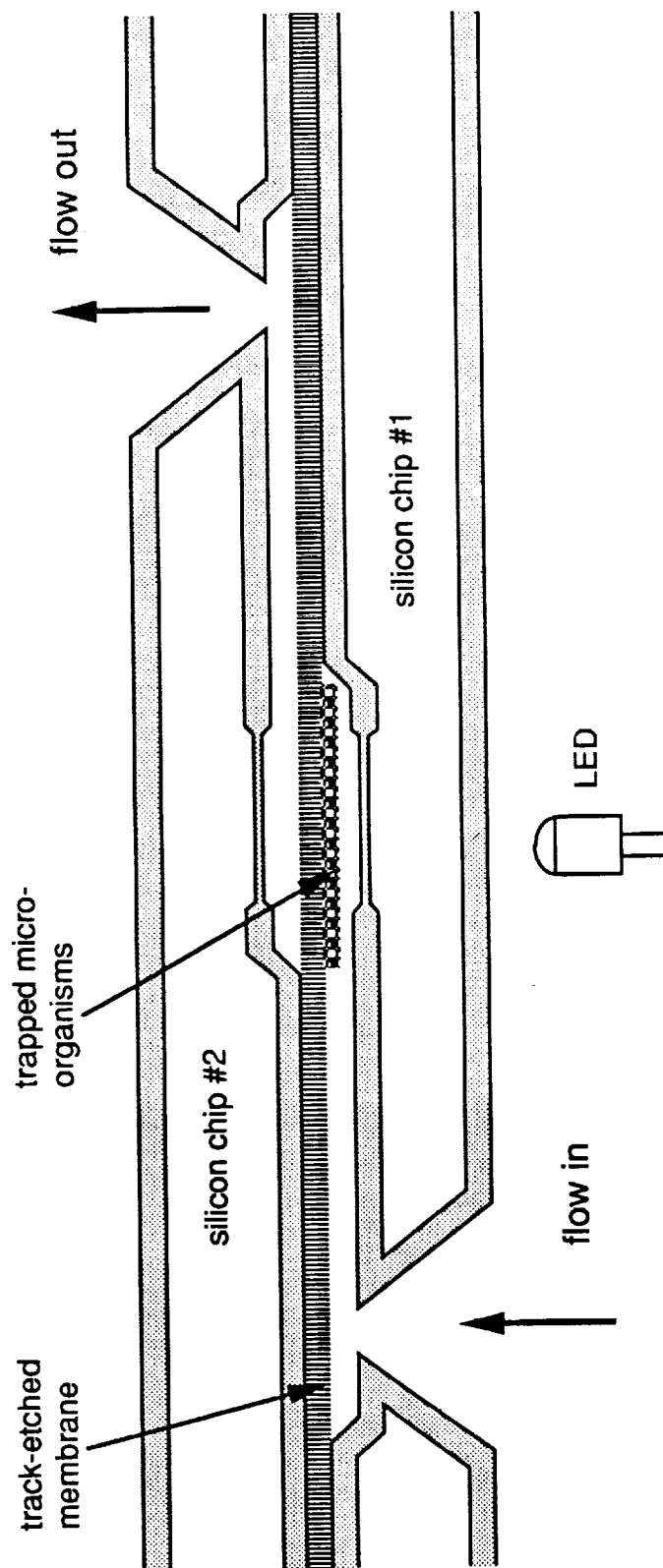Fluid
outlet

Figure 6.

179

Figure 7.

180

Figure 8.

Figure 9.

182
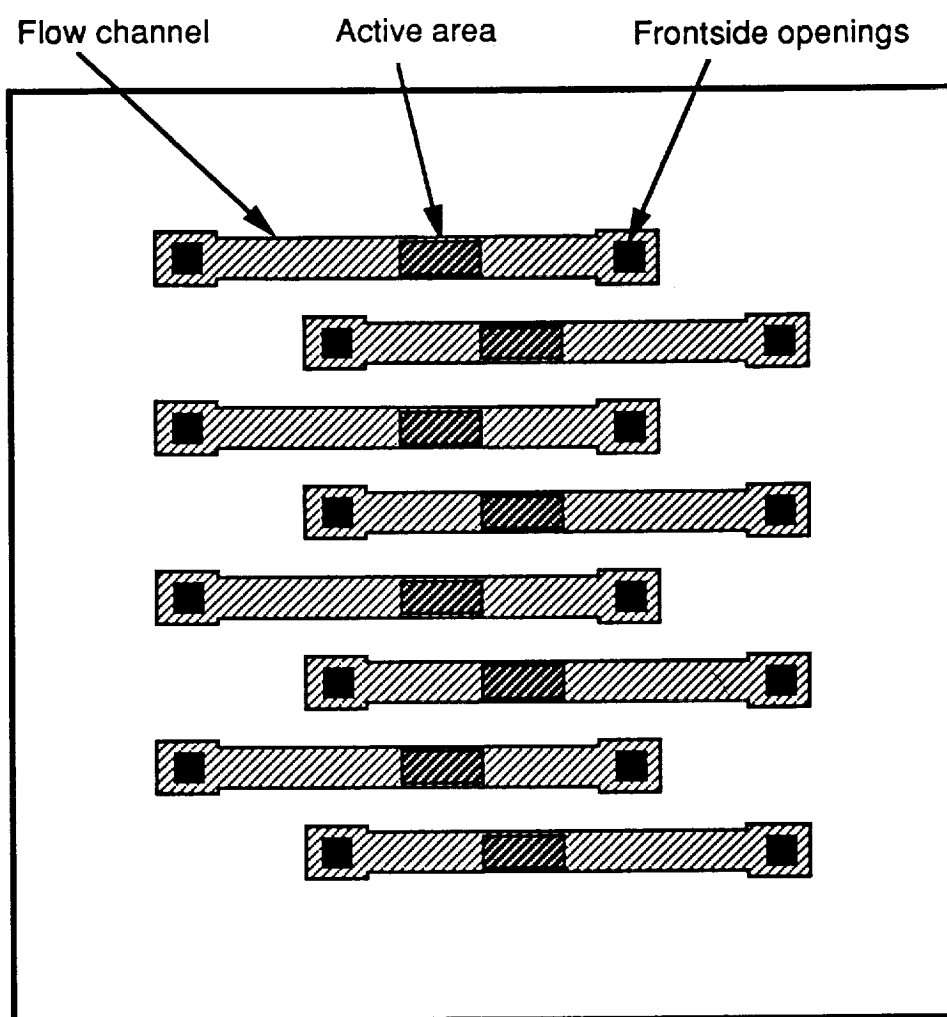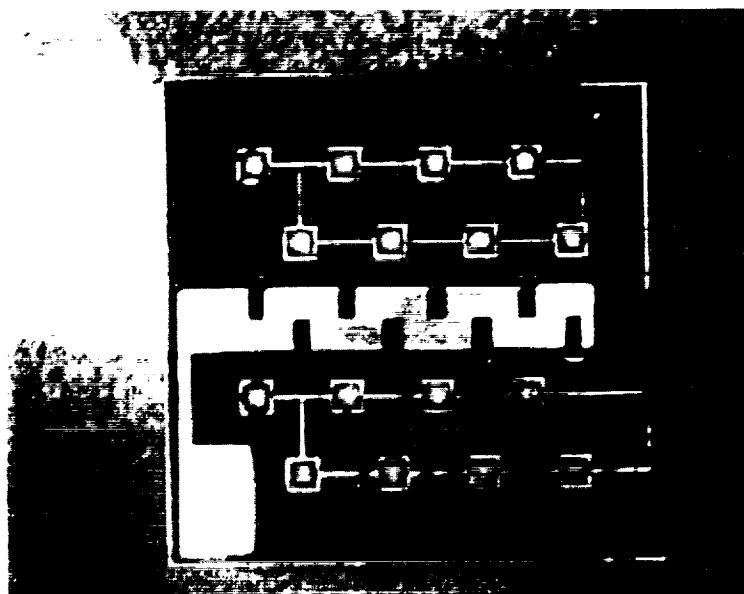
Microtechnologies
and
Applications to Space Systems Workshop

MICROROVERS

ROBOTIC VEHICLES FOR PLANETARY EXPLORATION

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ABSTRACT

Future missions to the Moon, Mars, or other planetary surfaces will use planetary rovers for exploration or other tasks. Operation of these rovers as unmanned robotic vehicles with some form of remote or semi-autonomous control is desirable to reduce the cost and increase the capability and safety of many types of missions. However, the long time delays and relatively low bandwidths associated with radio communications between planets precludes a total "telepresence" approach to controlling the vehicle. A program to develop planetary rover technology has been initiated at the Jet Propulsion Laboratory (JPL) under sponsorship of the National Aeronautics and Space Administration (NASA). Prototype systems with the necessary sensing, computing, power and mobility resources to demonstrate realistic forms of control for various missions have been developed and initial testing has been completed. These testbed systems, the associated navigation techniques currently used and planned for implementation, and long-term mission strategies employing them are all described in this talk.

Difficulties Inherent in Miniaturizing Current Rover Technologies for Use as Planetary Explorers

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Technologies currently under consideration for planetary rovers are intended for vehicles of a certain size. As the size of the rover decreases, certain difficulties arise, including:

- The locomotion characteristics change
- Some subsystems scale non-linearly
- Some subsystems don't scale
- Certain mission objectives are unachievable

The talk will focus on how these difficulties can arise. Not being aware of these difficulties can lead to improper designs based on the false assumption that the designs scale in some linear fashion.

MICROMACHINING TECHNOLOGIES FOR AUTOMOTIVE APPLICATIONS

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Abstract

The rapid advancement of micromachined mechanical devices is a direct result of innovative adaptations of the well-established integrated-circuit technology. The major motivation for research in this field is the potential applications in batch-fabricated integrated sensors and silicon-based microactuators. These devices promise new capabilities and improved performance-to-cost ratio over conventional hybrid-manufactured devices. Micromachined transducers that can be fabricated compatibly with an integrated circuit process are the building blocks for integrated microsystems with added functionality, such as closed-loop control and signal conditioning. These advantages are especially attractive in the highly cost- and quality-competitive automotive industry.

In advanced vehicle design, increase in sophisticated electronic controls is inevitable. Various automotive sensors and actuators are identified to be critical to future vehicle development. Bulk- and surface-micromachining based on single-crystal and poly-crystalline silicon have demonstrated great potential for satisfying automotive demands for low-cost, high-quality, and high-reliability microsensors and actuators. Several microfabricated sensors are described as examples to illustrate the applicability of micromechanics in the automotive industry.